IAFMM FISH OIL BULLETIN

international association of fish meal manufacturers

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RECOMMENDED METHOD OF ANALYSIS FOR DETERMINATION OF UNSAPONIFIABLE MATTER IN FISH OIL

1. Principle

Unsaponifiable matter includes those substances frequently found dissolved in fats and oils which cannot be saponified by the caustic alkalies but are soluble in the ordinary fat solvents. Included are the higher aliphatic alcohols, sterols, pigments, and hydrocarbons.

Applicable to fats and oils, especially those marine oils which contain more unsaponifiable matter than usually found in normal tallows and greases. Also applicable to vegetable oil, deodorizer distillates and sludges. Does not apply to feed grade fats.

2. Principle Reagent

All reagents shall be of analytical reagent quality.
Ethyl Alcohol, 95%
Ethyl ether, free from peroxides.
Acetone.
Concentrated aqueous potassium hydroxide solution, approximately 50% KOH by weight. Prepare by dissolving 60g KOH in 40 ml of water and cooling.
Aqueous potassium hydroxide solution; about 0.5N, prepare by dissolving 30g KOH in water, cooling and diluting to 1 litre.

Aqueous sodium hydroxide solution; 0.02N accurately standardized.
Phenolphthalein indicator solution, 1% in 95% alcohol.

3. Apparatus

Separating funnels, 250ml capacity.
Erlenmeyer flasks, narrow mouth, 200ml with 20/40 standard taper outer joint.
Condensers, with 20/40 standard taper joint to fit the 200ml Erlenmeyer flasks. Air condensers may be used.
Beakers, 250ml.
Erlenmeyer flasks, or flat bottom fat extraction flasks, 50ml.
Glass stoppered cylinders, about 300mm height and 35mm diameter to contain at least 150ml.
Glass siphon.

4. Method

Weigh accurately 2 to 2.5g of well mixed sample into the 200ml Erlenmeyer flask. Add 25ml of alcohol and 1.5ml of concentrated aqueous KOH solution (50%) and mix. Boil gently, with occasional swirling, under a reflux condenser for 30 minutes. No loss of alcohol should occur during saponification.
Transfer while warm to the extraction cylinder, washing with a total of 50ml of water. Wash the flask with 50ml of ethyl ether and add to the cylinder. Cool contents to room temperature (20^{o} to 25^{o}C).
Insert the stopper and shake vigorously, taking usual precautions to release any pressure that may develop in the cylinder.
Allow the layers to separate and clarify. Use a glass siphon to remove the upper layer as completely as possible and transfer the ether to a 250ml separating funnel containing 20ml of water.

Repeat the extraction two more times with 50ml portions of ethyl ether, shaking vigorously. Adjust the siphon after each separation in order to remove the ether layer as completely as possible.

Rotate the combined ether extracts gently with the 20ml of water. Violent agitation at this stage may result in troublesome emulsions. Allow the layers to separate completely and draw off the lower aqueous layer. Wash the ether twice more with the 20ml portions of water, shaking vigorously each time and discarding the lower aqueous layer after separation.

Wash the ethyl ether solution three times with 20ml portions of about 0.5N aqueous KOH, shaking vigorously each time, and follow each alkali treatment by washing with 20ml of water. If an emulsion forms during this washing procedure, allow to separate as much as possible, discard the clear aqueous layer, and proceed with the next step, leaving any emulsion in the separating funnel with the ether layer. After the third washing with the 0.5N KOH, wash the ether with successive 20ml portions of water until the washings are no longer alkaline to phenolphthalein.

Transfer the ether solution to a 250ml beaker, rinsing the separating funnel and its pouring edge with ether and adding the rinsing to the main solution. Evaporate to about 5ml and transfer quantitatively, with the aid of several small portions of ether, to a 50ml Erlenmeyer or fat extraction flask which has been previously dried and weighed. Place flask on a steam bath to remove the ether. When practically all of the ether has evaporated, add 2 or 3 ml of acetone and remove all solvent completely by passing a gentle current of clean dry air through the warmed flask. Complete the drying to constant weight, preferable in a vacuum oven at 75° to 80°C and an internal pressure of not more than 200mm of mercury. Cool in a desiccator and weigh.

After weighing, dissolve contents of flask in 2ml of ethyl ether and then add 10ml of 95% alcohol, previously neutralized to a faint pink colour, using phenolphthalein indicator. Titrate with 0.02N NaOH to the same final colour. Correct weight of residue for free fatty acid content (1ml of 0.02N NaOH is equivalent to 0.0056g of oleic acid). Also correct the weight of the residue for reagent blank obtained by conducting the determination in the same manner but omitting the oil or fat.

Note:

1. The extractions may be made in separating funnels if so desired.

2. Some fats high in unsaponifiable matter, particularly those of marine origin, may require more than three extractions for complete removal of the unsaponifiable matter. This is best judged by making another extraction and evaporating this separately. There should be less than 0.001g unsaponifiable matter in this extract.

5. Calculation

$$\frac{100(\text{wt. of residue} - \text{wt. of fatty acid} - \text{wt. of blank})}{\text{wt. of sample}} = \text{Unsaponifiable matter (\%)}$$

6. Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 0.2%.